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RESEARCH ARTICLE

The determination of effective concentration of acethonilic *Ipomoea cairica* leaves extract against laboratory and field strains of *Aedes albopictus* and *Aedes aegypti* mosquito larvae

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ABSTRACT

Inundated with escalating dengue outbreaks, there is an urgent call to find alternate potential vector control methods as the currently employed method fails to curb the expanding of dengue virus transmission in Malaysia. Supported by this aim, we are interested in exploiting the potential of *Ipomoea cairica* leaves extract towards primary and secondary vectors of dengue fever, *Aedes aegypti* and *Aedes albopictus*. To assess the effectiveness of this plant extracts towards *Aedes* larvae, we carried out two complementary analyses. First, we observed the comparative effectiveness of larvicidal activity *I. cairica* extract against the laboratory and field strains of *Ae. aegypti* and *Ae. albopictus*. Then, we determined the effective lethal dose of this plant extract against *Aedes* larvae using log-probit regression analysis of the SPSS 20.0 programme. Results from bioassay demonstrated that *I. cairica* leaves extract was highly effective to induce larvicidal mortality of *Ae. albopictus* and *Ae. aegypti* within 24 and 48 hours post-treatment. Results from the factorial analysis of variance (ANOVA) also indicated that there were significant differences in larvicidal activity between species and strains used ($P<0.05$). It is interesting to notify that the sequence of effectiveness for the larvicidal activities of *I. cairica* acethonilic leaves extract is in the manner; *Ae. albopictus* field strain > *Ae. aegypti* laboratory strain > *Ae. aegypti* field strain > *Ae. albopictus* laboratory strain. The *I. cairica* leaves extract displayed high larvicidal activity against *Ae. albopictus* as compared to *Ae. aegypti*. This is the first evaluation involving the comparison of *I. cairica* leaves extract effects for the laboratory strain and field strain of *Ae. albopictus* and *Ae. aegypti*.

Keywords: *Aedes albopictus*; *Aedes aegypti*; biological control; plant extracts; *Ipomoea cairica.*

INTRODUCTION

Over the last five decades, the cycles of dengue avenues have increased dramatically with a 30fold global incidence expanding geographically into many previously unaffected areas (WHO 2009). In an attempt to ward off epidemiology of dengue virus, a series of insecticide discoveries have been developed since the late 1930s as tools for mosquito control approaches (Becker *et al*., 2003). Due to the widespread of insecticide resistance, the control of the mosquito vector population currently had turned into a failure (Hasan *et al*., 2016) and insecticide resistance management is crucial (Dusfour, 2019). This factor has shifted researchers' attention on developing other alternative controls, including biological controls (Frentiu *et al*., 2014) as a very promising method to reduce the transmission of dengue whilst pose no risk to human health and the environments.

The recognition of mosquitocidal compounds in plant extracts has called many research to test the potential of phytochemicals as one of potential candidatures in biological control of *Aedes* mosquitoes. The compounds within plant parts such as phenolics, terpenoids, flavonoids and alkaloids have been proven to induce larvicidal and adulticidal properties (Elumalai *et al*., 2012). According to Alouni *et al*. (2009), results from phytochemicals studies could undoubtedly reduce the rampant usage of chemical insecticides and increase the opportunity of botanical pesticides for biological control of medically important vectors.

Research thrusts have focused on studying phytochemicals derived from genus *Ipomoea* since 1950s (Meira *et al*., 2012). *Ipomoea cairica* is diverging species from Family Convolvulacea, which commonly known as 'Railway creeper' due to their twining characteristics (Austin & Huáman, 1996;

Thiagaletchumi *et al*., 2014). The major constituents of *I. cairica* extracts consist of alkaloids, sterols, flavonoids, reducing sugars, tannins, saponins, terpenoids, anthraquinones, glycosides and phenols properties which could exhibit cytotoxic activity (Ralte, 2014).

Thomas *et al*. (2004) revealed the larvicidal efficacy of *I. cairica* essential oils could induce 100% mortality against four medically important vectors, *Culex tritaeniorhynchus*, *Ae. aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* at very low concentrations. The high potential of *I. cairica* as biolarvicidal agents were also proven against key dengue vectors, *Ae. aegypti* and *Ae. albopictus.* Results of these studies revealed the leaves part of *I. cairica* exhibited the strongest larvicidal activities against both principal vectors of dengue virus at low concentrations of LC_{50} and LC_{95} at 105.59 ppm and 321.56 ppm for *Ae. albopictus*, while for *Ae. aegypti* were at 101.94 ppm and 447.78 ppm, respectively (Ahbirami *et al*., 2014).

Complementing the need for biological control agents as alternative tools for mosquito controls in Malaysia, we are interested in determining the effective lethal concentration of *I. cairica* against *Ae. albopictus* and *Ae. aegypti* larvae. In this study, we compared the baseline concentration and the susceptibility of both Vector Control Research Unit (VCRU) laboratory and field strains of *Aedes* mosquitoes from urban and sub-urban regions in Penang towards *I. cairica*.

MATERIALS AND METHODS

Mosquito samples and rearing

Two sampling sites were chosen in this study. *Aedes aegypti* field strain was collected from a small urban residential area in Sungai Dua consisted of apartments and commercial areas (N 5.348508, E 100.300104) and known as dengue hotspot area (Mohiddin *et al*., 2015). While *Ae. albopictus* field strain was collected from a sub-urban area in Batu Maung (N5.283881, E100.279320) where this area surrounded by vegetation, playground and landed houses. Both sites were chosen due to the dominancy of *Ae. aegypti* and *Ae. albopictus* population within the respected area. In this study, we used F1 larvae of field strain mosquitoes. Two weeks prior to the experiment, eggs of both *Aedes* species were obtained by placing ovitraps with soaked paddles inside as an oviposition substrate in the selected sites. The collected larvae were reared in enamel trays contained with dechlorinated water and fed daily with 0.5 gm larval food made of dog biscuits, beef liver, yeast and milk powder at the ratio of 2:1:1:1. The larval culture was maintained at a temperature of 28±2°C and 70-85% humidity. After emergence, both adult *Aedes* species were morphologically identified to species using the taxonomic key by Becker *et al*. (2003). The emergence of colonies confirmed their dominancy within their respective areas. In order to ensure the uniformity of the larvae stage used in bioassays, we used newly hatched F1 larvae after the separation of adults at F0 generation collected from the fields. The larvae were reared and maintained until they reached a suitable stage for the continuation of bioassays.

Laboratory strains of *Ae. aegypti* and *Ae. albopictus* established by Vector Control Research Unit (VCRU), School of Biological Sciences, Universiti Sains Malaysia since 1970s were used in this study as the baseline susceptible strain. The eggs batches of the F1 generation were hatched in enamel trays soaked with dechlorinated water and maintained using the same procedures for field strain *Aedes* larvae mentioned above. All larvae strains and species were reared until the late third instar larva (L3), and early fourth instar larva (L4) stages before being subjected to bioassays test.

Collections and Preparations of *Ipomoea cairica* leaves

Fresh *I. cairica* leaves were collected around Sungai Dua area (N5°.351981, E100°.298572) Penang Island. As the leaves part of *I. cairica* showed the most remarkable larvicidal activities (Ahbirami *et al*., 2014), only leaves parts of this plant were segregated for the extraction preparations. The collected plants were identified and authenticated by a plant taxonomist from Herbarium Unit, Universiti Sains Malaysia. *I. cairica* leaves were air-dried until a constant weight was achieved for one to two weeks at room temperature condition. The dried leaves were mechanically ground into fine powders using a commercial electrical stainless-steel blender (Panasonic: MX-899TM).

Ipomoea cairica Soxhlet extraction

Ipomoea cairica leaves were extracted with Soxhlet apparatus using acetone as the solvent. As solvents influence the polarity of organic compounds are influenced by solvents, acetone was chosen as the crude solvents in this study due to their established reactions with *I. cairica* leaves (Ahbirami *et al*., 2014). A total of 40 g powdered *I. cairica* leaves were weighed using an analytical balance and inserted into a Soxhlet extraction thimble. Pebbles were added to optimize the flowing of solvent passing through the plant powder within extraction cycles. The thimble filled with plant powder was capped with cotton wool and loaded into the Soxhlet extractor. 2 L of acetone solvent was filled into round-bottom flask placed on the heating mantle. The boiling point for acetone extraction was set up at 50.5°C. The extraction cycles were run for 3 hours with a few flushing until the colour of solvent in the siphon side arms turned to almost clear. The procedure was repeated using different 40 g plant powder for each round. The excess solvent within round-bottom flask was loaded into several Petri dishes. Then, the Petri dishes were transferred to the oven at 37°C for drying purposes.

Stocks solution and serial concentrations preparation

To prepare the stock solutions, one gram of crude extract yield was weighed using an analytical balance. The weighed solid residues were then dissolved in 100 mL acetone to make up 10,000 ppm stock solutions. From prepared stock solutions, serial dilutions were then prepared by adding specific amounts of plant extracts from the stock solution into distilled water to get desired concentrations for the assay. The remaining stock solutions were kept in a refrigerator at 4°C until further used.

Larvicidal bioassay using *Ipomoea cairica* extract

The larvicidal bioassays for determining the effective activities of *I. cairica* extraction was run following the World Health Organization guidelines of mosquito larvicides (WHO, 2005). Twenty late third and early fourth instar larvae were introduced into testing cups filled with a 200 mL desired range of concentrations. A total of 12 concentrations were tested separately for each species and strains at 10 ppm, 30 ppm, 50 ppm, 70 ppm, 80 ppm, 100 ppm, 120 ppm, 150 ppm, 200 pmm, 300 ppm, 400 ppm and 500 ppm given a range of 0% to 100% mortality. Each concentration set was replicated three times. The control set consisted of 199 ml of distilled water added with 1 ml of 10% acetone. Throughout the testing period, no larvae food was offered (Ishak *et al*., 2014; Samuel *et al*., 2014) due to not disrupting the infection process through oral intake of *I. cairica* particles. After 24 h and 48 h exposure, the larval mortality was observed and recorded. Dead larvae

were identified as motionless larvae after probing with a needle in the siphon or cervical region (WHO, 2005).

Statistical Analysis

Statistical analysis was carried out to determine the lethal concentration (LC) and significant effects among the strains and species used subject to larval mortality. The efficacy of *I. cairica* extracts was determined using the LC_{50} and LC_{95} value, analyzed using log-probit regression analysis at 95% confidence interval in SPSS 20.0 programme. To detect the significant differences between different strains of *Aedes* species after the exposure to *I. cairica* extract, the factorial analysis of variance (ANOVA) was conducted. Prior to analysis, all the data were tested for normality (Shapiro-Wilk) to fulfil the assumption of ANOVA. Degree of concentrations, *Aedes* mosquito species and strains were subjected as fixed factors, whereas the larval mortality was considered as dependent variables. The level of significant differences for the statistical analyses was set at P<0.05.

RESULTS

Larvicidal effect of *Ipomoea cairica* against *Aedes* larvae

After 24 hours post-treatment, it was observed that the mortality response at the highest concentration of 500 ppm of both strains of *Ae. albopictus* has achieved 98%–100% mortality (Figure 1A), whereas, for *Ae. aegypti*, the mortality response of both strains were slightly lower, which were between 78% to 100% mortality (Figure 1C). In this study, larvicidal activity exhibited by *I. cairica* extracts towards *Ae. albopictus* were significantly higher than *Ae. aegypti* (F=7.656, *df*=1, P<0.05; Table 1). With respect to larval strains, the field strain of *Ae. albopictus* and laboratory strain of *Ae. aegypti* showed a faster mortality response as compared to others (Figures 1A & 1C) and was found to be statistically significant between each strain (F=5.513, *df*=1, P <0.05; Table 1). Both field strain of *Ae. albopictus* and laboratory strain of *Ae. aegypti* achieved 100% larval mortality after 24 hours post-treatment. Whereas laboratory strain of *Ae. albopictus* and field strain of *Ae. aegypti* were late in achieving 100% mortality and happened only after 48 hours post-treatment. As shown in Figure 1, the bioactivity potential of *I. cairica* was varied significantly for each concentration (F=71.921, *df*=12, P<0.05; Table 1). Maximum larval mortality observed in the promoted concentrations ranged from 300 ppm – 500 ppm to achieve 100% larval mortality within the treated period (Figure 1). No mortality was notified in control treatments.

The effective lethal dose of *Ipomoea cairica* against *Aedes* larvae

In this study, the LC_{50} of *I. cairica* leaves extracts treated against both *Aedes* larvae ranged between 31.85 ppm until 251.71 ppm. Whereas, LC_{95} values ranged between 121.57 ppm until 1613.00 ppm. After 24 h, it was observed that the probit values of *Ae. albopictus* laboratory strain (LC_{50}: 251.71 ppm; LC_{95}: 1613.00 ppm; Table 2) were higher than those for the field strain (LC_{50}: 53.46 ppm; LC_{95}: 307.01 ppm; Table 2). In contrast for *Ae. aegypti* larvae, the lowest LC_{50} and LC_{95} values obtained after 24h for the laboratory strain (LC_{50}: 124.17 ppm; LC_{95}: 728.18

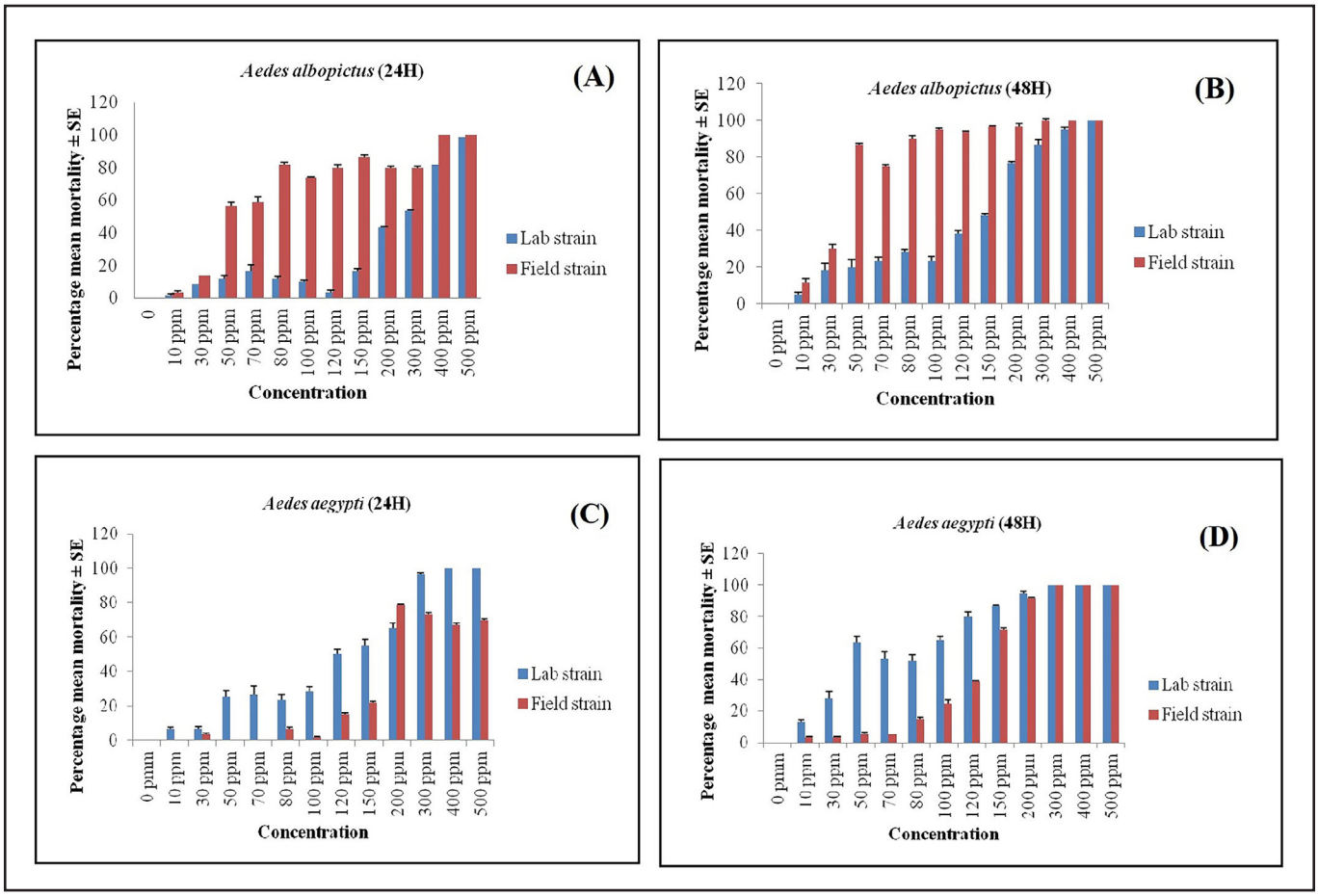

Figure 1. Mean percentage of *Aedes* larval mortality of; (A) *Aedes albopictus* at 24 hours post-treatment, (B) *Aedes albopictus* at 48 hours post-treatment, (C) *Aedes aegypti* at 24 hours post-treatment, and (D) *Aedes aegypti* at 48 hours post-treatment after exposure to different concentrations of *Ipomoea cairica* extract.

Table 1. Results from factorial analysis of variance (ANOVA) on the effect of larvae species, strains, days and serial conidial concentrations on larval mortality. Significant values are in bold. Data were log transformed prior to analysis

Source	*df*	Mean	*F*-value Square	*P*-value
Aedes species (A.S)	1	4.119	7.656	**0.006***
Strain (S)	1	2.966	5.513	**0.020***
Hours (H)	1	18.350	34.106	**0.000***
Concentration (C)	12	38.696	71.921	**0.000***
A.S*S	1	61.834	114.926	**0.000***
A.S*H	1	0.133	0.247	0.620
A.S*C	12	1.541	2.863	**0.001***
S*H	1	2.107	3.916	**0.049***
S*C	12	0.574	1.066	0.390
H*C	12	0.583	1.084	0.375
A.S*S* H	1	0.029	0.054	0.816
A.S*S*C	12	3.430	6.374	**0.000***
A.S*H*C	12	0.241	0.448	0.942
S*H*C	12	0.272	0.506	0.910
A.S*S*H*C	12	0.469	0.871	0.577

ppm; Table 2) as compared to the field strain (LC_{50}: 227.82 ppm; LC_{95}: 957.23 ppm; Table 2).

Referring to the probit analysis results, the order of larvicidal activity based on the LC_{50} and LC_{95} values and the effectiveness of *I. cairica* against both *Aedes* larvae were observed in the sequence of *Ae. albopictus* field strain > *Ae. aegypti* laboratory strain > *Ae. aegypti* field strain > *Ae. albopictus* laboratory strain (Tables 2 and 3). Based on this observation, it is evident from our understanding to speculate that field strain of *Ae. albopictus* were the most susceptible to *I. cairica* treatment as compared to other strain of larvae.

Deformities of *Aedes* larvae after *Ipomoea cairica* treatment

The morphological deformities that occurred in the treated larvae pointed out the potent bioactivity of *I. cairica* against both *Aedes* larvae used in this study (Figure 2). The extract treatment has caused massive morphological disruption with darkening and blackening of the midgut epithelial layer under light microscopy (Figure 2A). Meanwhile, the normal control larvae used in this study still retained their structure integrity (Figure 2B).

Table 2. The effective lethal dose of *Ipomoea cairica* against laboratory and field strains *Aedes albopictus* and *Aedes aegypti* treated for 24 hours and 48 hours

Species	Strain	Time (Hours)	LC_{50} ppm	LC_{95} ppm	Probit equation
Aedes albopictus	Laboratory	24H	251.71 (181.18–450.25)	1613.00 (746.15–10129.59)	2.039x–4.895
		48H	125.98 (93.09–179.89)	745.50 (409.47–2738.78)	2.130x–4.474
	Field	24H	53.46 (37.31–67.18)	307.01 (212.13–621.47)	2.167x–3.744
		48H	31.85 (21.12–41.82)	121.57 (86.25–231.75)	2.827x–4.250
Aedes aegypti	Laboratory	24H	124.17 (88.91–199.17)	728.18 (359.92–4904.70)	2.141x–4.483
		48H	49.60 (29.02–70.74)	355.99 (194.23–1663.15)	1.922x–3.258
	Field	24H	227.82 (183.50–285.33)	957.23 (625.42–2256.56)	2.638x–6.220
		48H	124.52 (112.45–140.62)	242.82 (199.57–340.49)	5.671x–11.882

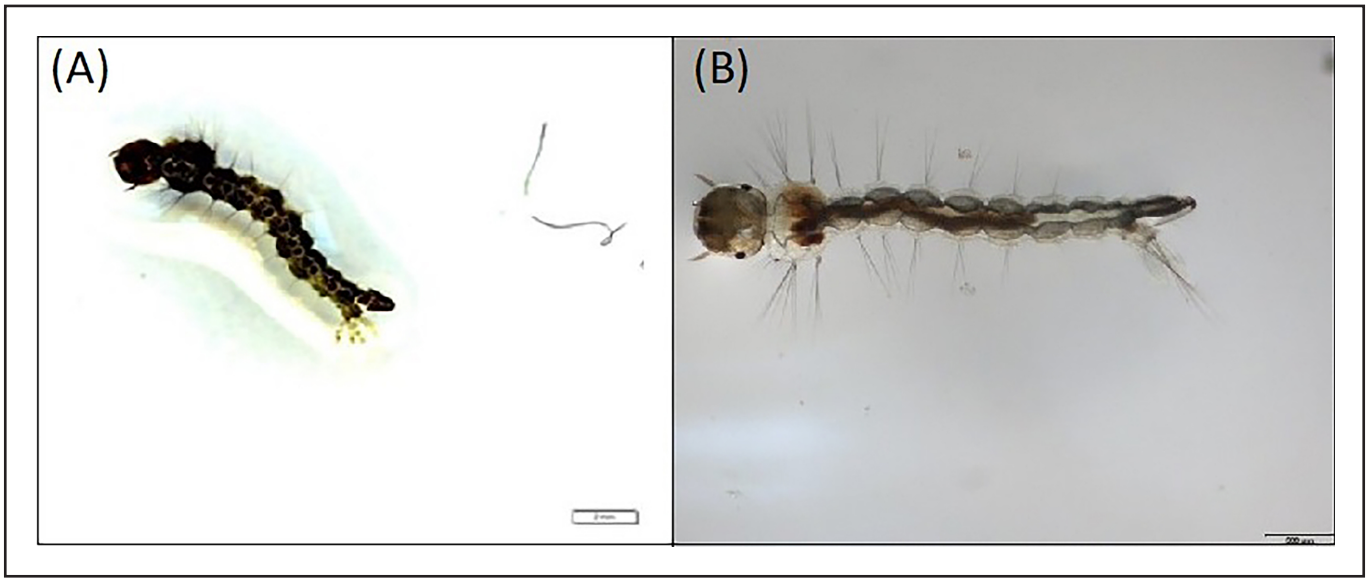

Figure 2. Morphological deformities exhibited by *Ipomoea cairica* leave extracts on treated larvae; (A) larvae with darkening abdomen, (B) normal larvae.

DISCUSSION

Following the sequential discoveries of bioactivity of plant extracts against mosquitoes, here we demonstrated the biological potential of *I. cairica* leaves extracts towards the laboratory and field strains of both medically important vectors (*Ae. albopictus* and *Ae. aegypti*). Based on our study, we had found that the bioactivity of *I. cairica* leaves extracts caused can induce 100% larval mortality on both laboratory and field strains of *Aedes* larvae at as low as 300 ppm. It was found that the *Ae. albopictus* field strain is the most susceptible strain to *I. cairica* extract compared to other treated groups used in this study. Justifying the ability of *I. cairica* extracts to induce mosquito larvae, the effectiveness of *I. cairica* crude extracts against mosquito larvae have also been proven against *Cx. tritaeniorhynchus*, *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* in the previous study (Thomas *et al*., 2004). The results from the previous study and the present observation ratifying the potential of *I. cairica* as an alternative larvicide to fight off *Aedes* larvae in Malaysia.

In this study, the bioactivity of *I. cairica* leaves extracts showed various larvicidal activity reflecting upon the dosage of concentration being applied in the treatments. Naturally, the mortality rate was increased, corresponding to the rises in the concentration of the plant extracts used in the treatment (Samuel *et al*., 2014). Results of *I. cairica* larvicidal activity against *Aedes* larvae in this study also showed a positive correlation with the plant extracts concentration used. A similar trend was observed when the larvicidal properties of *Knema attenuata* extracts were tested against *Ae. albopictus* and *An. stephensi,* the mortality of the treated larvae showed was in a concentration-dependent manner. In facts, the geographical origin of the plant itself also plays important roles in the variation of phenolic composition and antioxidant activities of the plant extracts (Guo *et al.,* 2011; Liu *et al.,* 2018). Goa *et al*. (2011) indicated that the phenolic contents were influenced by growing conditions; where low temperature enhancing the synthesis of phenylalanine ammonia-lyase (PAL) in plants and may increase the production of phenolics, while high altitude and extended sunlight hours with higher UV radiation positively affect the activity of phenolics synthase (Kishore *et al*., 2010). Ishak *et al*. (2014) also showed a much lower concentration to cause 85% until 100% larvae mortality using *I. cairica* leaves extracts, even though those larvae were the same VCRU strain used in the current study. This hypothesizes that the bioactivity of plant extracts is influenced by the geographical origins of the plants and is associated with the dosage applied to the treatments.

The maximum efficacy of the plant extraction also depends upon the polarity of the solvents used to selectively extract the target phytochemical compounds (Samuel *et al*., 2014). The molecular affinity of polar solvent will extract polar molecules, whereas a non-polar solvent will extract non-polar molecules (Rawani *et al*., 2010). Since the polarity of our targeted compound are alkaloids and flavonoid, which usually performed using alcoholic solvent, we chose acetone as a solvent used in this present study. Besides, the preliminary information conducted by Ahbirami *et al*. (2014), also revealed the best solvent extract that is suitable to increase the bioactivity of *I. cairica* against *Aedes* larvae is acetone rather than methanol. This is due to the moderately low polarity index of acetone (5.1 indexes), which had conversely impacts on high efficacy of bioactivity of compounds which also had been proven in our results. The impact of solvent on the effectiveness of plant extract can be seen between our study and Ishak *et al*. (2014). Despite finding the correct solvent to extract the active target molecules, selecting an appropriate solvent is also important to obtain the maximum yield of crude extract. As the physiochemical properties of acetone have a lower viscosity, the extraction using acetone as solvent could provide a better maximum high yield (Rathod & Rathod, 2014), thus justifying acetone as a suitable solvent to be used in the present study.

The pattern of the mortality rate might be varied according to the larval species and strain. In this study, patterns of mortality rate observed on *Ae. albopictus* field strain showed faster larvicidal effect even at the slightest different amount of concentration being applied to the treatment. It was found that the LC_{50} and LC_{95} values obtained for *Ae. albopictus* field strain was the lowest, indicating a potent larvicidal effect of *I. cairica* leaf extracts towards this strain. The differences in the bioactivity of plant extracts towards different larval species and strain, possibly due to active ingredients of plant extracts that attack various aspects of the immune defence mechanism of the larvae which depends on their local adaptation. In this present study, the collected field strain of *Ae. aegypti* was from the urban area, whereas *Ae. albopictus* was from the sub-urban area. As *Ae. aegypti* predominantly live within or near to the house due to its selective preferences on human blood, the infestations of the dengue virus commonly occur within the urban area. Our selected study site for *Ae. aegypti* collection is Sungai Dua, Penang, which is known as one of the hotspot areas in Penang Island, Malaysia (Ritchie, 2014; Mohiddin *et al*., 2015). The extensive usage of insecticides during fogging activities in that area after cases notification probably has created insecticide resistance in *Ae. aegypti* population used in this study. A survey conducted by Sumayyah *et al*. (2016) to the residents living in Sungai Dua area reported that they currently experienced the effects of intense use of mosquito insecticide, which had lowered the mortality effect on mosquitoes within that area. This might probably explained that the lower susceptibility of field strain *Ae. aegypti* towards the current tested control method which using plant extracts rather than insecticides control since the population never been exposed to plant extracts.

Since then, the mode of action of these *I. cairica* leaves extract on mosquito larvae is still lacking. However, it is known that the phytochemical compounds in plant extracts are formed by the secondary metabolites mechanism, which potentially caused the toxic effects effect to the insect physiology in many ways (Ghosh *et al*., 2012; Ralte, 2014). In our study, we observed the morphological deformities formed in the treated *Aedes* larvae, such as having darkening and blackening at their abdomen. The observation made during the assay also showed abnormal motions of larvae such as coiling and convulsion at the bottom of treated containers. This abnormal motion showing the earlier symptoms of toxicant activity of *I. cairica* extract against *Aedes* larvae in the present study before death took place.

Results from the phytochemical screening of *I. cairica* extracts revealed that the presence of alkaloids, saponins, tannins, and flavonoids were detected in the leaves extract of *I. cairica* (Ishak *et al*., 2014). Tannins are one of the metabolite compounds that act as an inhibitor to proline-rich protein preventing the process of protein synthesis of the cell from taking place (Ralte, 2014), thus causing depression on growth-rate and inhibition of the digestive enzyme (Bennick, 2002). The digestive tract of most of the insects is lined with a peritrophic membrane which plays an important roles in the digestive process and provides the protection from invasion by viruses, bacteria and other

pathogens (Liu *et al*., 2014). As the peritrophic membrane composed of chitin and proteins, the action of tannins present in the *I. cairica* leaves extract, it could possibly cause the disruption of peritrophic membrane formation inside the guts of *Aedes* larvae. This disruption could inhibit larval development and lead to mortality which probably explained the cause of larval mortality in the present study. Besides, another phytochemical constituent of plant extracts that could cause the toxin to *Aedes* larvae is alkaloids. Alkaloids are known to have a toxic effect on insects and vertebrates by affecting the Acetylcholinesterase (AChE) action. The inhibition of AChE will cause acetylcholine accumulation at the synaptic pathway, disturbing the nerve impulse transmission (Rattan, 2010). Corresponding to this event, the insects exposed to alkaloids will finally have a lack of coordination in their body systems (Hussein *et al*., 2018), including physiological stress (Muñoz, 2020) and eventually death, as observed in the recent study.

Despite many research being conducted to find the most bio-potential plant extracts to inhibit the mosquito larval development, however, up to our knowledge, there is still no comparative studies conducted on the effectiveness of this plant extract against both laboratory and field strains of *Aedes* larvae. Interestingly, the *I. cairica* leaves crude extract used in this study showed the effectiveness towards the field strain of *Ae. albopictus* and *Ae. aegypti*. Despite the different level of susceptibility in both *Aede*s larvae shown in this study, it is worth to note that *I. cairica* leaves extracts to have a high potential to serve as an alternative bio-larvicidal agent. It is a bonus to discover that there is still available botanical origin that can be used as safer larvicides which may act as a suitable alternative product to ward off *Aedes* populations in Malaysia.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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